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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Philip A.	Beachc	Baltimore, MD

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

WIDESPREAD REQUIREMENT FOR LIGAND-STIMULATED HEDGE-HOG PATHWAY ACTIVITY - DIGESTIVE TRACT TUMORS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* _____ ☐ CD(s), Number _____

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☐ Application Data Sheet. See 37 CFR 1.76

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Respectfully submitted,

SIGNATURE _____ Date 7/15/03

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JHU Ref. No.: JHU-4283

Widespread Requirement for Ligand-Stimulated Hedgehog Pathway Activity in Growth of Digestive Tract Tumors

Inventor: Philip A. Beachy

Widespread requirement for ligand-stimulated Hedgehog pathway activity in growth of digestive tract tumors

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Activation of the Hedgehog (Hh) signaling pathway by sporadic mutations or in familial conditions, such as Gorlin Syndrome, has been associated with tumorigenesis in skin, cerebellum, and skeletal muscle (reviewed in refs. 1,2). Here we demonstrate that a wide range of digestive tract tumors, including the majority of those originating from esophagus, stomach, biliary tract, and pancreas, but not from colon, displayed elevated levels of Hh pathway activity, which were suppressed by the Hh pathway antagonist cyclopamine. Cyclopamine also suppressed cell growth in vitro and caused regression of xenograft tumors in vivo. Unlike Gorlin syndrome tumors, pathway activity and cell growth in these tumors is driven by endogenous expression of Hh ligands, as indicated by presence of *Sonic* and *Indian hedgehog* transcripts, by pathway- and growth-inhibitory activity of a Hh-neutralizing antibody, and by the dramatic growth-stimulatory activity of exogenously added Hh ligand. Our results identify a group of common lethal malignancies in which Hh pathway activity, essential for tumor growth, is activated not by mutation but by ligand expression.

The Hedgehog (Hh) signaling pathway specifies patterns of cell growth and differentiation in a wide variety of embryonic tissues. Mutational activation of the Hh pathway, whether sporadic or in Gorlin Syndrome, is associated with tumorigenesis in a limited subset of these tissues, predominantly skin, cerebellum, and skeletal muscle[1,2]. Known pathway-activating mutations include those that impair the ability of Patched (PTCH, the target of Gorlin Syndrome mutations), a transporter-like Hh receptor[3], to restrain Smoothened (SMO) activation of transcriptional targets via the Gli family of latent transcription factors (reviewed in refs. 1,2,4,5) Binding of Hh is functionally equivalent to genetic loss of PTCH, in that pathway activation by either requires activity of SMO, a seven transmembrane protein that binds to and is inactivated by the pathway antagonist, cyclopamine [6]. The recent finding that pathway activity plays a role in growth of a significant proportion of small cell lung cancers [7], a tumor type not associated with Gorlin Syndrome, suggested the possibility that other non-Gorlin tumors may also require Hh pathway activity for growth. We investigate here the role of pathway activity in tumors derived from the gut, a tissue with prominent and diverse roles for Hh signaling in developmental patterning and tissue homeostasis [8-10].

We began our examination of gut-derived tumors by assaying for expression of *Sonic* and *Indian hedgehog* (*SHH* and *IHH*), which encode members of the Hh ligand family that are expressed in early endoderm and throughout gut development [9,11]. We detected *SHH* and *IHH* mRNA in 37 of 38 cell lines (97%) from esophagus, stomach, biliary tract, pancreas, and colon carcinomas (Fig. 1). The Hh target genes *PTCH* and *GLI* were co-expressed in most cell lines from esophagus (4/6), stomach (6/6), pancreas (5/6), and biliary tract (5/9) tumors, but not in those derived from colon (0/11). The expression of pathway targets in cells that also express Hh ligands suggests the autonomous operation of an active signaling process within several types of digestive tract tumors.

Autonomous pathway activity was confirmed by the high-level expression of luciferase activity from an exogenously introduced Hh-inducible reporter [12] in all cell lines producing detectable *PTCH* mRNA (Fig. 2a). Hh pathway activity in these cell lines furthermore was inhibited in a dose-dependent manner by the Hh pathway-specific antagonist cyclopamine, and not by tomatidine, an inactive but structurally related compound (Fig 2a) [13]. These results suggest that high levels of Hh pathway activity may

be a common feature of digestive tract tumors, prompting us to further investigate a pathway role in tumor growth. We found that cyclopamine treatment inhibited growth of tumor cell lines from esophagus, stomach, biliary tract, and pancreas by 75 to 95% as compared to tomatidine controls (Fig. 2b). Strikingly, significant growth inhibition was observed only in tumor lines expressing *PTCH* mRNA, confirming that the effects of cyclopamine treatment were pathway specific rather than generally cytotoxic.

Because the properties of cell lines adapted to long term growth *in vitro* may not accurately reflect those of tumors growing *in vivo*, we also examined pathway activation in freshly resected stomach and pancreatic tumors by measuring endogenous *PTCH* mRNA levels. For each specimen, RNA for quantitative RT-PCR analysis was isolated from ten consecutive 10 μM cryosections, after histologic analysis of both immediately flanking sections to determine tumor content. We found, as compared to adjacent normal tissue, that *PTCH* mRNA levels were elevated 23-371 fold in stomach tumors (average = 129; n = 9) and 69-5044 fold in pancreatic tumors (average = 448; n = 15) (Fig. 3a).

To examine the role of Hh pathway activity in growth we analyzed pancreatic carcinomas passaged once as xenografts in nude mice then cultured and immediately assayed *in vitro*. Of six such xenografts four expressed *PTCH* mRNA (data not shown), including a matched pair of primary and metastatic tumors from a single patient. All four of these *PTCH*-expressing primary xenografts expressed Gli reporter in a cyclopamine-sensitive manner (Fig. 3b). Cyclopamine treatment of these *PTCH* mRNA-expressing xenografts also resulted in decreased viable cell mass (Fig. 3c), demonstrating more extreme cell-killing effects of Hh pathway blockade than observed in established tumor cell lines (see Fig. 2b). In contrast, single passage xenografts lacking *PTCH* mRNA grew equally well in control and cyclopamine containing media (Fig. 3c), again confirming that cyclopamine effects were pathway specific rather than generally cytotoxic.

To examine the effects of cyclopamine treatment *in vivo*, subcutaneous xenografts were established from HuCCT1, a metastatic cholangiocarcinoma cell line. After growth to an average size of 180 mm^3, mice bearing these tumors were injected daily with cyclopamine, resulting in complete or near complete regression of all nine treated tumors within 14 days (Fig. 3d,e). Control vehicle-treated tumors, in contrast,

continued growing. As previously reported [7,14], all mice survived cyclopamine treatment without obvious adverse reactions. These results demonstrate specific *in vivo* tumoricidal effects of Hh pathway blockade by treatment with cyclopamine.

These findings establish widespread activation of the Hh pathway in gut-derived tumors, and further demonstrate a role for pathway activity in tumor cell growth, *in vitro* and *in vivo*. Yet Gorlin Syndrome is not associated with a higher incidence of gut-derived tumors, and *PTCH* mutations in these tumors have not been reported, suggesting a non-mutational mechanism for pathway activation. Given the expression of *SHH* and *IHH* mRNA in nearly all gut-derived tumors examined, we investigated the role of Hh ligand binding in pathway activity. We measured Hh-inducible reporter activity in HuCCT1 cholangiocarcinoma cells treated with 5E1 monoclonal antibody [15], which binds Shh and Ihh ligands [16] and blocks signaling by disrupting ligand binding to Ptch [17]. Autonomous activation of transfected reporter was not affected by control antibody, but was dramatically reduced by incubation with 5E1 at 0.1 or 10 μg/ml (Fig. 4a). Reporter activity in contrast was augmented ~8 fold by addition of purified Shh ligand to 25 nM (Fig. 4a). Addition of 5E1 in combination with Shh ligand reduced reporter activity to a level intermediate between those seen with either reagent alone (Fig. 4a), indicating a mutual antagonism between 5E1 and ligand in activation of pathway.

Reporter activity in cells from single passage pancreatic cancer xenografts was also antagonized by 5E1(Fig. 4b). Treatment with 5E1 antibody dramatically reduced viable cell mass as well (Fig. 4c), and both this cell-killing effect and the reporter effect were observed exclusively in cells from tumors that expressed endogenous *PTCH* mRNA. We further investigated the relationship between ligand concentration and growth by adding 5E1 antibody to cells from a single passage pancreatic tumor xenograft at a level just sufficient to block growth. We then added Shh protein and found that growth correlated positively with increasing concentrations (Fig. 4d). Rates of growth from this experiment plotted as a function of Shh concentration (Fig. 4e) indicate that ligand-induced pathway activation is rate limiting and that unperturbed growth of these cells is sub-maximal.

Hh ligand and 5E1 blocking antibody are mutually antagonistic in their effects on reporter activity, and produce opposite effects on growth of cells from these gut-

derived tumors (Fig. 4a-e), thus revealing a Hh ligand-dependent mechanism for pathway activation and cell growth. In sharp contrast, neither addition of Hh ligand nor of 5E1 blocking antibody significantly affected growth of cells from a single passage pancreatic tumor xenograft that did not express *PTCH* mRNA (Fig. 4f), demonstrating the specificity of antibody and ligand effects. We also observed no significant ligand- or antibody-induced change in growth of medulloblastoma cells derived from a mouse model of Gorlin Syndrome (Fig. 4f), in which Hh pathway is activated through loss of Ptch function [14,18]. In contrast to the antibody-resistant xenograft cells, however, the medulloblastoma-derived cells require pathway activity for growth and can be killed by cyclopamine treatment [14].

Ligand-independent mutational activation of the Hh pathway has been linked to the formation of tumors associated with Gorlin Syndrome, such as medulloblastoma. Despite a widespread activation of and dependence on the Hh pathway for medulloblastoma growth [14], however, only a fraction of sporadic tumors can be assigned to pathway-activating mutations, suggesting that other mechanisms of pathway activation may often be at play. Here we establish such a mechanism by demonstrating that pathway activation and growth of cells from a group of commonly lethal gut-derived malignancies is ligand-dependent. Small cell lung cancer (SCLC), also arising from endodermally derived epithelium and associated with Hh ligand expression, recently has been linked to transient reactivation of the Hh pathway within the airway epithelium for regulation of progenitor cell fates during injury repair [7]. A similar role for Hh signaling in renewal of the epithelium of the gut and its derivatives is suggested by embryonic and adult expression of the Hh pathway targets *Ptch* and *Gli* (ref. 9; K.B. and D.N.W, unpublished data) and by the requirement for Hh signaling for stem cell proliferation within the murine gut epithelium [9]. Whether renewal of injured gut epithelium is associated with transient Hh pathway reactivation is not known, but it is notable that increased rates of esophageal, gastric, and pancreatic carcinomas occur in association with acid injury in Barrett esophagus, in *Helicobacter pylori* infection, or with exposure to alcohol, cigarette smoke, and certain dietary components [19-21].

Exposure to such factors likely causes injury to the gut epithelium, eliciting a chronic state of injury repair and a consequent increase in proliferative stem or progenitor cells that may arise through ligand-dependent reactivation of the Hh pathway. Many of these agents are also mutagenic, thus potentially enhancing tumor formation by subjecting an enlarged pool of stem or stem-like target cells to potentially oncogenic mutations. However induced, our results identify a group of common and frequently lethal gut-derived tumors, readily diagnosed by their expression of endogenous pathway targets such as *PTCH*, which may respond to antagonist- or antibody-mediated pathway blockade, even in advanced stages of metastatic disease.

METHODS

Cells and tissues. Origins and sources are described in the supplementary table. First passage pancreatic cancer xenografts were derived from freshly harvested pancreaticoduodenectomy specimens as described [14]. The ability of these xenografts to represent pancreatic tumors in the general population is indicated by our experience that approximately 65% of specimens yielded xenografts (data not shown). After reaching 25 mm in greatest dimension, xenograft tumors were harvested, minced, and plated into tissue culture vessels in RPMI, 20% Fetal Bovine Serum (FBS) for assays as described below. The diagnosis of frozen samples from gastric and pancreatic adenocarcinoma resections and adjacent normal stomach and pancreas was microscopically confirmed by two pathologists (D.M.B. and A.M.) and RNA was prepared as described [14].

RT-PCR. Total RNA was prepared from frozen sections or from tissue culture monolayers using RNAwiz reagent according to the manufacturer's instructions. cDNA was synthesized from 1μg of total RNA in a 33 μl reaction using You-Prime First-Strand Beads (Amersham Pharmacia, Piscataway, NJ) and random hexamers. PCR reactions were performed using 10% of the first strand reaction and oligonucleotide primers specific for the cDNAs of interest for 38 cycles of 1 min. each at 94C, 55C, and 72C followed by a single 15 min. incubation at 72C. For all primer pairs, specificity was confirmed by sequencing of PCR products. For quantitative RT-PCR, 10% of the

first strand reaction was amplified using IQ-SYBR Green Supermix, an i-cyclerIQ real time detection system (Bio-Rad, Hercules, CA) and specific oligonucleotide primers for PTCH or PGK. Amplification was performed at 95 C for 5 minutes followed by 40 cycles of 10, 15, and 30 seconds at 95C, 55C and 75C respectively. Bio-Rad software was used to calculate threshold cycle (C_T) values for *PTCH* and for the housekeeping gene, phosphoglycerate kinase (PGK). For each sample, *PTCH* expression was derived from the ratio of *PTCH* to *PGK* levels using the formula $2^{-\Delta CT}$ where $\Delta C_T = C_{T\text{-}PTCH} - C_{T\text{-}PGK}$. *PTCH* levels in tumors were presented as a ratio to levels detected in adjacent normal tissue (Fig. 3a).

Hh-responsive reporter assays. Hh-responsive firefly luciferase and control SV-40 Renilla luciferase reporter assays were performed on subconfluent triplicate cultures as described [22]. Two days after transfection, cells were cultured for two days in assay media: RPMI-1640 (Bio-Whittaker, Walkersville, MD) supplemented with 0.5% (established cell lines) or 20% (first passage xenografts) FBS and containing combinations of 5E1 anti-Hh monoclonal antibody, recombinant doubly lipid modified Sonic Hedgehog (ShhNp) peptide[12], cyclopamine purified from *Veratrum* extract, or tomatidine (ICN, Costa Mesa, CA) at the concentrations indicated in the text.

Proliferation assays. Cells were cultured in triplicate in 96 well plates in assay media to which 5E1 MAb, ShhNp, and/or cyclopamine was added at 0 hours at concentrations indicated in the text. Viable cell mass was determined by optical density measurements at 490 nm ($O.D._{490}$) at 2 and 4 days using the CellTiter96 (Promega, Madison, WI) colorimetric assay. Relative growth was calculated as O.D.(day 4) – O.D.(day 2)/O.D.(day 2).

Xenograft treatment. HUCCT1 tumors (n=18) were grown in athymic (nude) mice to $180mm^3$ and treated with cyclopamine (50mg/kg/day, subcutaneous injection) or control vehicle as described[14].

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Supplementary Information accompanies the paper on *Nature*'s website (http://www.nature.com).

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Figure 1. Widespread expression of transcripts encoding Hh pathway components in digestive tract tumor cell lines. RT-PCR products demonstrating expression of genes encoding Hh pathway ligands (*SHH* and *IHH*) and target genes (*PTCH* and *GLI*) in tumor cell lines from sites in diagram (left). Red bars (right) indicate the percent of tumor cell lines expressing detectable *PTCH* mRNA at each site.

Figure 2. Cyclopamine suppression of Hh pathway activity and growth in digestive tract tumor cell lines correlates with expression of *PTCH* mRNA. ***a***.

Normalized activity of transiently transfected Hh-responsive luciferase reporter and dose-dependent suppression by the Hh pathway antagonist cyclopamine. ***b.*** Change in tumor cell viability measured by MTS (soluble tetrazolium salt) assay after culture in 3.0 μM cyclopamine or tomatidine (control). (Abbreviation: Bil: biliary)

Figure 3. Hh pathway activity and requirement for growth of tumor cells *in vivo*. ***a.*** Elevated *PTCH* mRNA in surgically resected pancreatic and gastric carcinomas was detected by quantitative RT-PCR and normalized to adjacent normal stomach (n=10) and pancreas (n=1). ***b.*** Normalized Hh-responsive reporter activity and suppression by 3.0 μM cyclopamine in first passage pancreas carcinoma xenografts. ***c.*** Corresponding reduction in viable tumor cells upon culture with 3.0 μM cyclopamine. Note that reduced viability is observed exclusively in xenograft lines with elevated Hh pathway activity. ***d.*** Change in human HuCCT1 human choloangiocarcinoma xenograft tumor volume in mice treated for 14 days with vehicle (control; n = 9) or cyclopamine (n = 9). ***e.*** Representative photographs of control- and cyclopamine- treated mice. Note full regression of tumor in lower right.

Figure 4. Ligand dependence of Hh pathway activity and growth in digestive tract tumors. ***a.*** Mutually antagonistic effects of Hh ligand and blocking antibody on activity of a Hh reporter. The Hh neutralizing 5E1 monoclonal antibody suppresses and Shh ligand increases reporter activity in HuCCT1 cells. Combined addition of antibody and ligand produces intermediate effects, depending on relative concentrations. ***b.*** Hh reporter activity in first passage

pancreas carcinoma xenografts and dose-dependent suppression with 5E1 MAb. ***c.*** MTS assay demonstrating reduced viability corresponding to Hh pathway suppression by 5E1 MAb. ***d.*** MTS assay showing growth (in arbitrary units) of PX184 first passage *PTCH* mRNA expressing pancreas xenograft cells cultured in control antibody (dashed line) or with 5E1 MAb at a level just sufficient to suppress growth (0.1 µg/ml; solid lines), and with the indicated concentrations of added Shh ligand. ***e.*** Growth rate (in arbitrary units) of PX184 cells from data in (*d*). Dashed line represents growth rate of cells cultured with control antibody. ***f.*** Modulation of cell growth rate by 5E1 MAb and Shh ligand in single passage pancreatic xenografts (PX-184, PX169) and medulloblastoma cells ($PZp53^{MED1}$). Note opposite responses to ligand and antibody of PX-184 cells, which express *PTCH* mRNA, and the lack of response of PX-169 and $PZp53^{MED1}$ cells, which respectively lack detectable Hh pathway activation, or display constitutive pathway activation due to lack of functional PTCH [14].

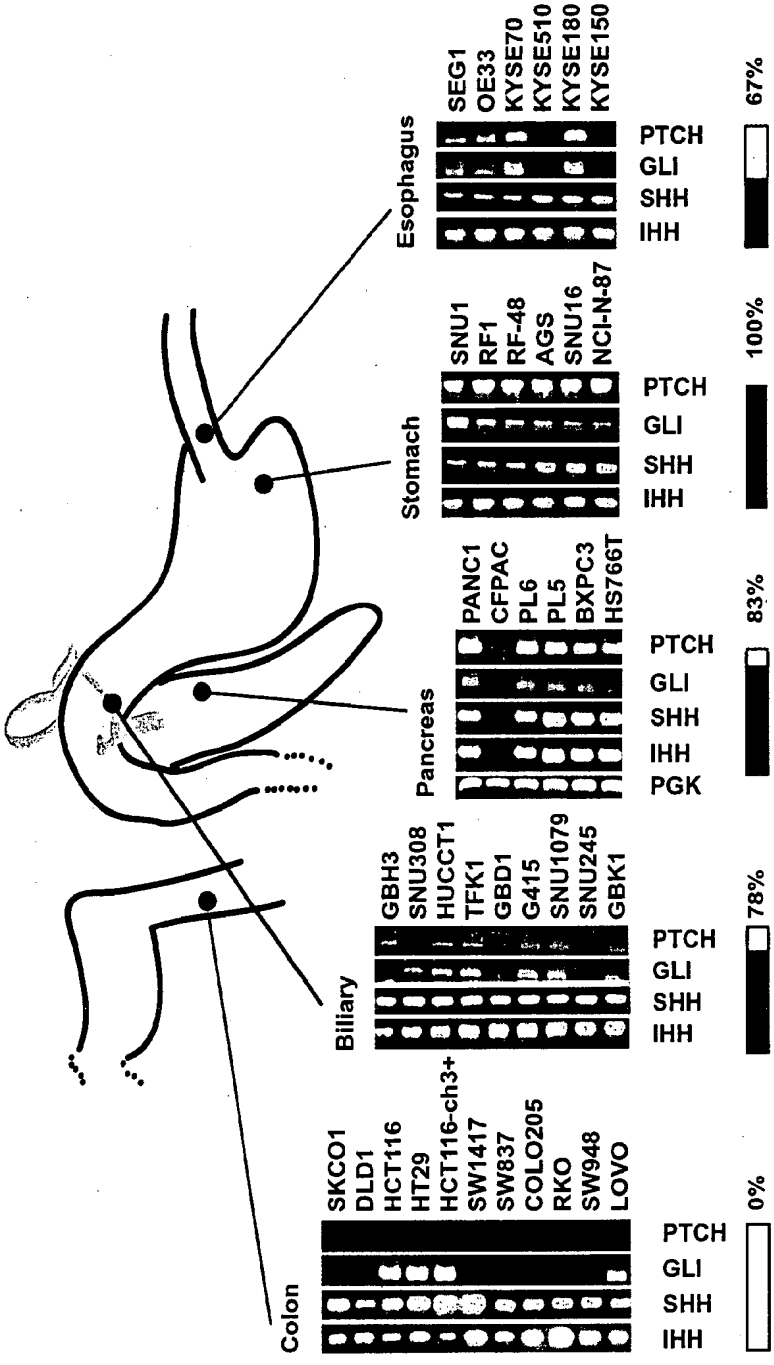

Figure 1
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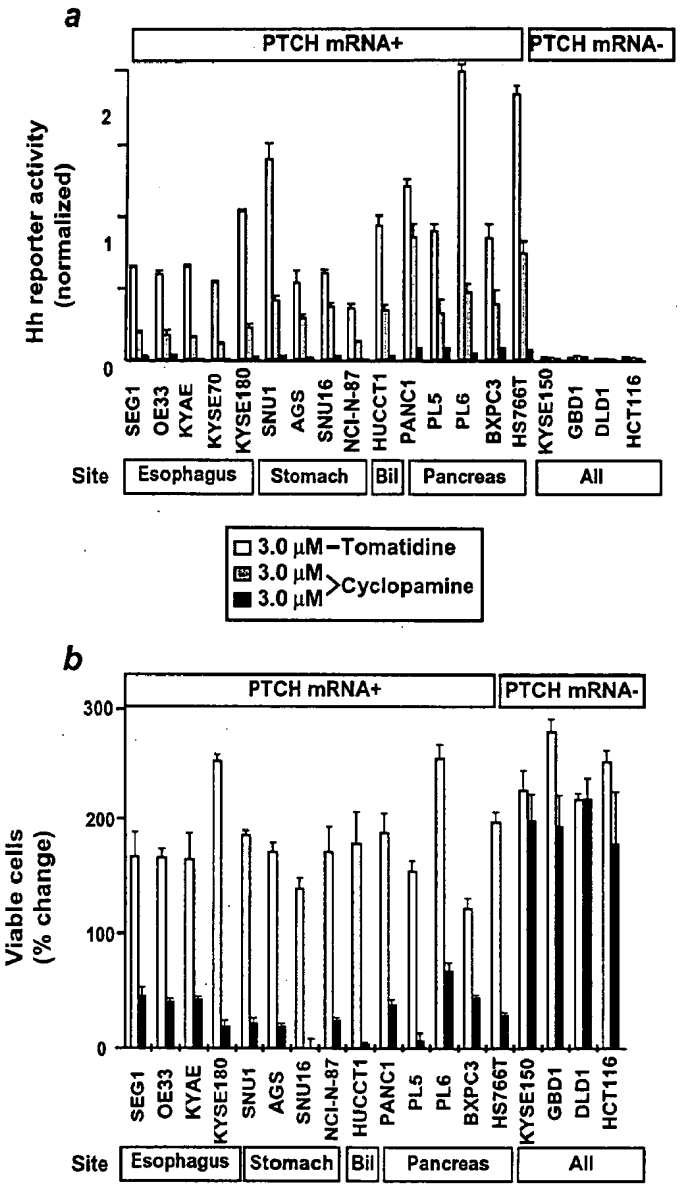

Figure 2
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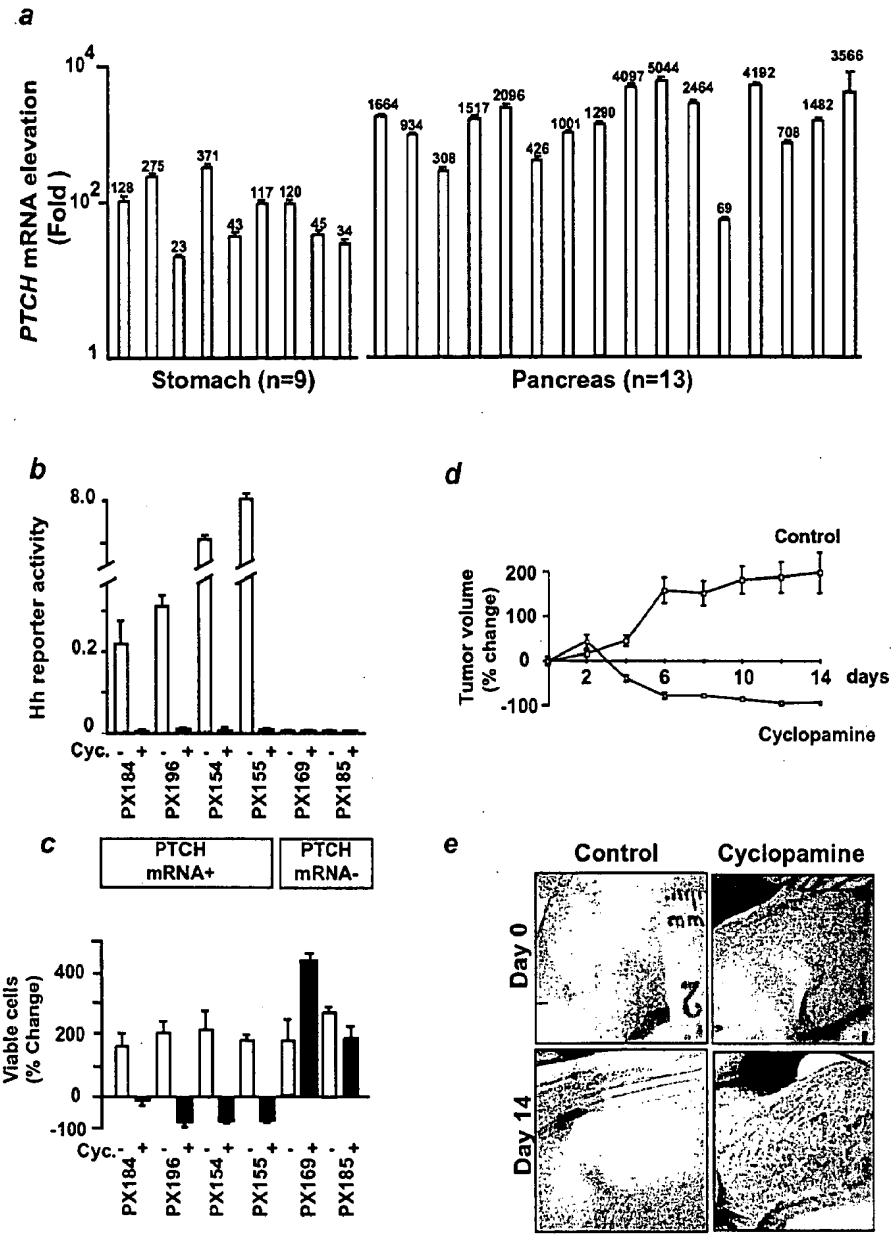

Figure 3
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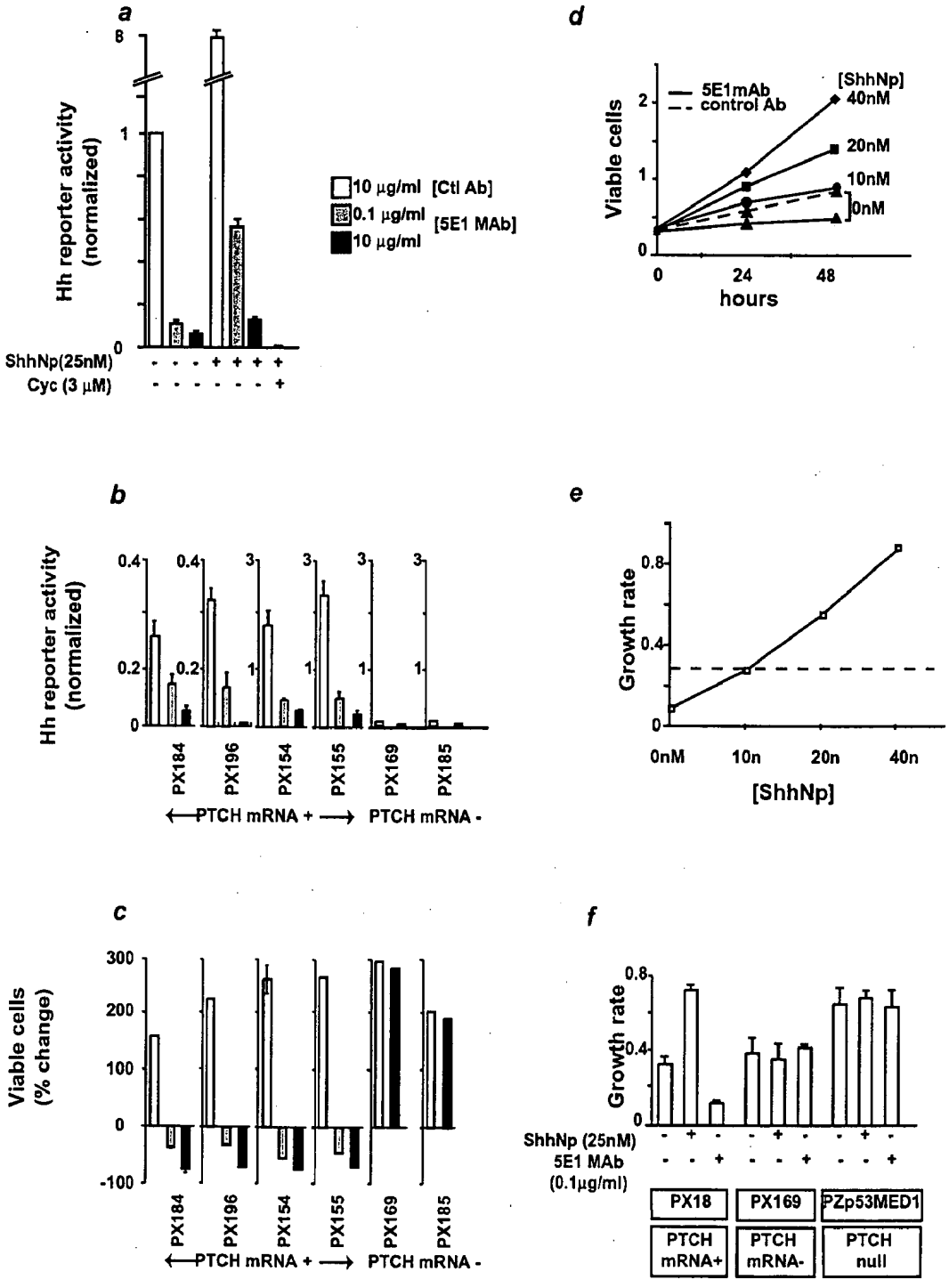

Figure 4
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DESIGNATION	TUMOR	HISTOLOGY	GRADE	Sample type	Stage	REF./ SOURCE
SEG1	ESOPHAGUS	ADENOCA	---	Cell Line	Pri.	1
OE33	ESOPHAGUS	ADENOCA	---	Cell Line	Pri.	2
KYAE	ESOPHAGUS	ADENOCA	---	Cell Line	Pri.	3
KYSE70	ESOPHAGUS	SQUAMOUS	---	Cell Line	Pri.	3
KYSE510	ESOPHAGUS	SQUAMOUS	---	Cell Line	Pri.	3
KYSE180	ESOPHAGUS	SQUAMOUS	---	Cell Line	Pri.	3
KYSE150	ESOPHAGUS	SQUAMOUS	---	Cell Line	Pri.	3
NCI-SNU1	STOMACH	ADENOCA	High (Signet ring)	Cell Line	Pri.	4
NCI-SNU16	STOMACH	ADENOCA	High (Signet ring)	Cell Line	Pri.	4
NCI-N-87	STOMACH	ADENOCA	Low	Cell Line	Met.	4
RF1#	STOMACH	ADENOCA	High (Signet ring)	Cell Line	Pri.	4
RF48 #	STOMACH	ADENOCA	High (Signet ring)	Cell Line	Met.	4
AGS	STOMACH	ADENOCA	Moderate	Cell Line	Pri.	4
PrGas 1	STOMACH	ADENOCA	Moderate	Snap-frozen	Pri.	5
PrGas 2	STOMACH	ADENOCA	High (Signet ring)	Snap-frozen	Pri.	5
PrGas 3	STOMACH	ADENOCA	High (Signet ring)	Snap-frozen	Pri.	5
PrGas4	STOMACH	ADENOCA	Moderate	Snap-frozen	Pri.	5
PrGas 5	STOMACH	ADENOCA	High (Signet ring)	Snap-frozen	Pri.	5
PrGas 6	STOMACH	ADENOCA	High (Signet ring)	Snap-frozen	Met.	5
PrGas 7	STOMACH	ADENOCA	High (Signet ring)	Snap-frozen	Pri.	5
PrGas 8	STOMACH	ADENOCA	High (Signet ring)	Snap-frozen	Pri.	5
PrGas 9	STOMACH	ADENOCA	Moderate	Snap-frozen	Pri.	5
PrGas 10	STOMACH	ADENOCA	High (Signet ring)	Snap-frozen	Pri.	5
SNU308	GALLBLADDER	ADENOCA	Moderate	Cell Line	Pri.	6
SNU1079	BILE DUCT	ADENOCA	Moderate	Cell Line	Pri.	6
SNU245	BILE DUCT	ADENOCA	Moderate	Cell Line	Pri.	6
HUCCT1	BILE DUCT	ADENOCA	High	Cell Line	Met.	7
TFK1	BILE DUCT	ADENOCA	Moderate	Cell Line	Pri.	8
GBD1	GALLBLADDER	ADENOCA	---	Cell Line	Met.	9
G415	GALLBLADDER	ADENOCA	---	Cell Line	Pri.	10
GBH3	GALLBLADDER	ADENOCA	---	Cell Line	Pri.	11
GBK1	GALLBLADDER	ADENOCA	---	Cell Line	Pri.	11
PANC1	PANCREAS	ADENOCA	---	Cell Line	Pri.	4
HS766T	PANCREAS	ADENOCA	---	Cell Line	Met.	4
PL6	PANCREAS	ADENOCA	---	Cell Line	Pri.	12
PL5	PANCREAS	ADENOCA	---	Cell Line	Pri.	12
BXPC3	PANCREAS	ADENOCA	---	Cell Line	Pri.	4
CFPAC1	PANCREAS	ADENOCA	---	Cell Line	Pri.	4
PX154	PANCREAS	ADENOCA	Moderate	Xenograft	Pri.	5
PX155	PANCREAS	ADENOCA	Moderate	Xenograft	Met.	5
PX169	PANCREAS	ADENOCA	High	Xenograft	Pri.	5
PX183	PANCREAS	ADENOCA	Moderate	Xenograft	Pri.	5
PX184	PANCREAS	ADENOCA	Moderate	Xenograft	Pri.	5
PX185	PANCREAS	ADENOCA	High	Xenograft	Pri.	5

PX196	PANCREAS	ADENOCA	High	Xenograft	Pri.	5
PrPanc 1	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
PrPanc 2	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
PrPanc 3	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
PrPanc 4	PANCREAS	ADENOCA	Moderate	Snap-frozen	Pri.	5
PrPanc 5	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
PrPanc 6	PANCREAS	ADENOCA	Moderate	Snap-frozen	Pri.	5
PrPanc 7	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
PrPanc 8	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
PrPanc 9	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
PrPanc 10	PANCREAS	ADENOCA	Moderate	Snap-frozen	Pri.	5
PrPanc 11	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
PrPanc 12	PANCREAS	ADENOCA	Moderate	Snap-frozen	Pri.	5
PrPanc 13	PANCREAS	ADENOCA	Moderate	Snap-frozen	Pri.	5
PrPanc 14	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
PrPanc 15	PANCREAS	ADENOCA	High	Snap-frozen	Pri.	5
SKCO1	COLON	ADENOCA	—	Cell Line	Pri.	4
D2D1	COLON	ADENOCA	—	Cell Line	Pri.	4
HCT116	COLON	ADENOCA	—	Cell Line	Pri.	4,14
HT29	COLON	ADENOCA	—	Cell Line	Pri.	4
SW1417	COLON	ADENOCA	—	Cell Line	Pri.	4
SW837	COLON	ADENOCA	—	Cell Line	Pri.	4
COLO205	COLON	ADENOCA	—	Cell Line	Pri.	4
RKO	COLON	ADENOCA	—	Cell Line	Pri.	4
SW948	COLON	ADENOCA	—	Cell Line	Pri.	4
LOVO	COLON	ADENOCA	—	Cell Line	Pri.	4
PZp53-MED1	CEREBELLUM	MEDULLO.	—	Cell Line	Pri.	13

NOTES:

Abbreviations: ADENOCA, adenocarcinoma; Pri., primary; Met., metastasis.

HuCCT1 and NCI-N-87 were established from ascitic fluid (i.e., metastatic cholangiocarcinoma and gastric ADENOCA, respectively), while GBD1 and HS766T were established from nodal metastases of a gallbladder and pancreatic ADENOCA, respectively. The remaining cell lines/xenografts are established from Pri. tumors, with the exceptions listed below. RF-48 is derived from the ascitic fluid (i.e., metastatic gastric ADENOCA) of the same patient from whom RF-1 is derived. PX155 was established from a lymph node Met. arising from the same patient from whom PX154 is derived. HCT116 and HCT116+ch3 (Fig. 1) are isogenic colon cancer cell lines except that the latter contains an extra copy of chromosome 3[14].

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All publications, patents and patent applications disclosed herein are incorporated into this application by reference in their entirety.

For example: "Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (volumes I-III) 1989, Cold Spring Harbor Laboratory Press, USA", "Harlowe and Lane, Antibodies a Laboratory Manual 1988 and 1998, Cold Spring Harbor Laboratory Press, USA" and "Ausubel *et al.*, Current Protocols 2001, John Wiley and sons, Inc." provide sections describing methodology for antibody generation and purification, diagnostic platforms, cloning procedures, etc. that may be used in the practice of the instant invention.